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#### REMARKS

Claims 1-32 are pending in the instant application. Claims 1-32 have been rejected. Claims 1, 24, 25, 29 and 31 have been amended. No new matter has been added by this amendment. Reconsideration is respectfully requested in light of the following remarks.

#### I. Priority

The Examiner suggests that the priority statement of the instant specification should be amended to indicate that the instant application is the U.S. National Stage of PCT/US00/15783, filed June 8, 2000, which claims priority to U.S. Provisional Patent Application 60/138,068, filed June 8, 1999. Applicants have made the suggested amendment and further corrected the serial number of the referenced provisional patent application.

#### II. Objections to the Claims

Claim 25 has been objected to because part b) reiterates the phrase "of claim 24" already present in part a). In accordance with the Examiner's suggestion, Applicant has deleted "of claim 24" in part b) of claim 25.

Claim 29 has been objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim should refer to other claims in the alternative only. The Examiner has interpreted claim 29 part b) as if the phrase "of claim 1" was deleted. Applicant has amended claim 29 part b) to remove reference to claim 1.

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### **III. Rejection of Claims Under 35 U.S.C. §112**

Claims 1-32 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

Specifically, claim 1 has been rejected because it does not make clear what the heterologous DNA segment is intended to be. As indicated at page 16, lines 4-18, a heterologous DNA segment is an identifiable segment of the construct that is not found in association with the construct in nature. Thus, claim 1 has been amended to indicate that the heterologous DNA segment comprises the components listed in part a) and b).

Claim 24 has been rejected for the recitation of "substantially homologous" when referring to DNA sequences being integrated at a pre-determined location of a genome. Applicant has amended claim 24 to omit the word "substantially".

Claim 29 has been rejected for being incomplete for omitting essential steps. The Examiner suggests that while the claim is drawn to activation tagging of a plant genome, no step indicates when the activation tagging takes place. In accordance with the teachings of the paragraph bridging pages 4 and 5, Applicant has amended claim 29 and 31 to indicate that activation tagging is carried out using a transcriptional promoter.

In light of the claim amendments, it is respectfully requested that the rejection of claims under 35 U.S.C. §112, second paragraph, be withdrawn.

Claims 24-32 have also been rejected under 35 U.S.C. §112, first paragraph, because while the specification is enabling for a DNA construct for integration of heterologous DNA segments into

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genomes, wherein the DNA construct is adapted for integrating a heterologous DNA segment at a pre-determined location in the *Chlamydomonas* genome, and a method for inserting a heterologous DNA molecule into a pre-determined location of a *Chlamydomonas* genome, does not reasonably provide enablement for DNA constructs adapted for integrating a heterologous DNA segment into pre-determined locations of other plant genomes, or methods for inserting a heterologous DNA into a pre-determined location in other plant genomes, or a method of activation tagging of a plant genome. The Examiner suggests that given that the specification teaches a prophetic example for homologous recombination in plants and that homologous recombination is not known to occur in plant species other than *Chlamydomonas*, as evidenced by the teachings of Puchta ((2001) *Plant Mol. Biol.* 48:173-182) and Terada et al. ((2002) *Nature Biotech.* 20:1030-1034), that the present specification is not enabling for the claimed methods.

Further, the Examiner suggests that, as written, claim 29 does not provide the essential step of expressing genes in the host genome to cause activation tagging and therefore the claim is not enabled. The Examiner suggests that the host plant is likely to have numerous insertions of the DNA construct and that numerous genes would be activated. Moreover, it is suggested that it is unclear how one skilled in the art would use a DNA construct of claim 24 in the method of activation tagging of claim 29, even if the specification enabled one skilled in the art to insert heterologous DNA into a pre-determined location by homologous recombination in any plant genome. The Examiner suggests that the heterologous DNA construct would only insert into the pre-determined location, and it is unclear how it would

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insert randomly into the genome and activate expression of genes in the genome. Applicant respectfully traverses this rejection.

Puchta teaches that while the frequency of gene targeting in plants is low compared to that of mammals, that several instances of gene targeting in flowering plants have been reported. Further, this reference teaches efficient gene targeting in the plant model system *Physcomitrella patens* (a moss). Tereda et al. teach two reports of successful targeting of endogenous natural genes in *Arabidopsis* (a dicot). This reference indicates that one of reported targeting procedures had not been repeated (i.e., Miao and Lam (1995) *Plant J.* 7:359-365; PTO-1449 reference AB) and that the other report detected the occurrence of undesirable events. Tereda et al. further teach a method for efficient gene targeting by homologous recombination in rice (a monocot).

MPEP 2164.02 indicates that an applicant need not have actually reduced the invention to practice prior to filing if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation. *In re Borkowski*, 422 F.2d 904, 908, 164 USPQ 642, 645 (CCPA 1970).

The specification as a whole, the Figures, and Example 1 at page 22 provides considerable direction and guidance to one of skill in the art as to the selectable markers, transposons, and screening protocol to produce and identify the desired recombinants. As evidenced by the teachings of Miao et al. and Puchta, methods needed to practice the invention were well-known as homologous recombination had been shown to occur in plants such as *Arabidopsis* and *P. patens*. A lack of validation of the experiments of Miao et al. in the literature, does not indicate

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that these methods are invalid, it merely indicates that similar experiments have not been published as of yet. Therefore, one of skill in the art would be readily able to apply the teachings of the instant specification to the teachings of Miao et al. and Puchta to generate a plant having a heterologous DNA segment inserted into pre-determined location or activation tagging of a plant.

MPEP 2164.06 states that the test of enablement is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (citing *In re Angstadt*, 537 F.2d 489, 502-04, 190 USPQ 214, 217-19 (CCPA 1976)).

As indicated *supra*, Puchta teaches several instances of gene targeting in flowering plants; however, the frequency of gene targeting was low. Thus, while the identification of a homologous recombinant or activation tagged flowering plant may require the screening of a large number of plants, this would not be considered an undue amount of experimentation in the field of plant biology as this type of screening is routine in the art and the specification teaches how one can readily screen for the desired recombinant (see page 26, lines 4-22).

MPEP 2164.05(a) indicates that, in general, the examiner should not use post-filing date references to demonstrate that the patent is non-enabling. Exceptions to this rule could occur if a later-dated reference provides evidence of what one skilled in the art would have known on or before the effective filing

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date of the patent application. *In re Hogan*, 559 F.2d 595, 605, 194 USPQ 527, 537 (CCPA 1977).

Thus, while the teachings of Tereda et al. have been improperly used to suggest the disclosure is non-enabling, it is a reference submitted to *Nature Biotech* at the time of filing and published four months after the filing of the instant application that further confirms that homologous recombination does occur in plants and further establishes that the level of skill in the art at the time of filing was quite high.

Regarding claim 29, as indicated *supra* this claim has been amended to indicate that activation tagging is accomplished using a DNA construct containing a transcriptional promoter to transactivate expression of a plant gene near the site of insertion of the DNA construct in the genome. Further, claim 29 is drawn to activation tagging of a plant genome to create variants displaying a desired phenotype. While there may be the occurrence of multiple insertions of the DNA construct into the plant genome, there may also be single insertion events and claim 29 is not limited to a single insertion event. In fact, a pleiotropic effect may be required to achieve a desired phenotype as multiple genes may be contributing to the phenotype (e.g., genes of a biosynthetic pathway).

Moreover, as would be appreciated by one skilled in the art, the use of a DNA construct of claim 24 in the method of activation tagging of claim 29 would be useful in altering the regulation of a specified gene. For example, replacement of an inducible promoter with a constitutive or tissue-specific promoter would have a profound effect on the expression of the coding region residing downstream. As claim 29 does not read on

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random insertion events, the use of a DNA construct of claim 24 would facilitate the insertion of heterologous DNA into a pre-determined location by homologous recombination in the plant genome.

Thus, in view of the teachings of the prior art and the high level of skill in the art for generating homologous recombinant monocot, dicot, and moss plants, the present application provides sufficient guidance in its description of constructs and screening methods for one of skill to use the constructs and methods of the present invention in methods of generating and identifying plants having a heterologous DNA segment inserted into pre-determined location or activation tagging of a plant. Withdrawal of this rejection is therefore respectfully requested.

#### **IV. Rejection of Claims Under 35 U.S.C. §103**

Claims 1-23 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Yoder et al. (WO 92/01370) in view of Hashimoto et al. ((1999) *Plant Sci.* 141:175-181) and Lloyd et al. ((1994) *Mol. Gen. Genet.* 242:653-657). The Examiner suggests the Yoder et al. teach methods to introduce heterologous DNA into genomes using transposon systems comprising substrate sites recognized by a transposase, selection and marker genes, and heterologous genes of interest wherein the transposon system can be introduced into plants by any means including *Agrobacterium* in which the transposon system is within the borders of the *Agrobacterium* tDNA. The Examiner acknowledges that Yoder et al. fail to teach the use of the cytosine deaminase (coda) gene.

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It is suggested that Hashimoto et al. teach the use of cytosine deaminase as a negative selection marker in plants and assert its use with transposon mobilization studies.

The Examiner further suggests that Lloyd et al. teach a strategy for using a recombinase system to remove a DNA segment containing the coding sequence of the GUS marker gene from the genome of plants, wherein substrate sites for the recombinase were placed between the GUS coding sequence and the CaMV 35S promoter that was operably linked to it such that the action of the recombinase removed the GUS coding sequence, but not the promoter.

The Examiner suggests that it would have been obvious at the time of filing to modify the method of Yoder et al. by using any other selection marker gene, such as the cytosine deaminase gene as taught by Hashimoto et al., as Hashimoto et al. asserts the use of cytosine deaminase in conjunction with transposons. It is suggested that it would have been obvious to place the cloning sites anywhere outside of the transposase substrate sites in the construct of Yoder et al., including the termini of the construct, to facilitate introduction of the genes of interest. It is further suggested that it would have been obvious to place polylinker sites within the transposase substrate sites, to facilitate insertion of further selection, marker or other heterologous genes. The Examiner also suggests that it would have been obvious to modify the DNA construct by inserting a marker gene, such as the GUS gene, within the construct such that a transposase substrate site was in between the promoter and marker coding sequence, following the strategy used by Lloyd et al. given that Lloyd et al. demonstrate this strategy provides

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another tool to monitor the excision event following the action of the transposase, as the promoter controlling expression of the marker gene would no longer be operably linked to the coding sequence. Applicant respectfully traverses this rejection.

Yoder et al. teach a DNA construct for integration into a plant genome, wherein the DNA construct contains a transposon and ancillary nucleic acid sequences. The construct can further contain a positive selection gene to identify cells having the ability to grow on a medium containing a particular antibiotic. Nowhere does this reference teach or suggest the use of a more than one selectable marker or the use of a negative selection gene.

Hashimoto et al. teach a DNA construct containing a positive and negative selection gene. However, the use of transposons as suggested by Hashimoto et al. is to study mutational events within the cytosine deaminase gene to identify cells that are able to grow even in the presence of the selective drug. See page 180, column 2, lines 1-12. In other words, the cytosine deaminase gene is a target for the transposon, not a component of a transposon-based DNA construct.

Lloyd et al. teach a DNA construct containing a promoter (CaMV 35S) and positive selection gene (hygromycin resistance gene) disrupted by two recombination target sites flanking a dispensable sequence (2.8 kb of plasmid sequence). Upon insertion into a plant genome, the recombination target sites and dispensable sequence are excised such that the promoter becomes operably associated with the positive selection gene allowing for selection of transformed cells. See Figure 1, page 654. This reference does not teach a DNA substrate for a selected transposase

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located between a promoter and coding sequence for a detectable marker gene such that following the action of the transposase, the promoter controlling expression of the marker gene would no longer be operably linked to the coding sequence. Quite to the contrary, this reference teaches that the promoter controlling expression of the marker gene would be operably linked to the coding sequence following the action of the transposase thereby transcriptionally activating the detectable marker.

MPEP 2143 indicates that to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. Further, *the teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure.* In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Because Yoder et al. do not teach the salient feature of a negative selection gene and Hashimoto et al. do not teach the use of a negative selection gene in a transposon-based construct, there is no suggestion or motivation within these references to modify or combine their teachings. Further, Lloyd et al. fail to teach the limitation set forth in claim 1 part b). It is only within the view of the instant specification that one of skill in the art would combine the heterologous DNA segments of claim 1 in

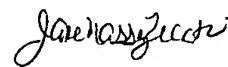
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the orientation outlined therein to arrive at the claimed invention. It is therefore respectfully requested that this rejection be withdrawn.

**V. Conclusion**

The Applicant believes that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Respectfully submitted,



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